ACCOMPLISHMENTS

This Berkeley Lab effort has already made significant progress toward achieving its goal. To date, the team has developed innovative robotic microscope and computer informatics technologies for documenting the uptake and retention of labeled AS-ONs in living cells (Figure 3). These technologies include:

- Visual servoing optical microscopy (VSOM), a remote robotics system that is the subject of a U.S. patent application, is being transferred to industry via a recently awarded 2002 California State BioSTAR grant. VSOM is being used not only to design and screen labeled AS-ONs, but also to develop "delivery vehicles" or "carriers" that help AS-ONs penetrate cells and reach gene targets throughout the body.
- A new bioinformatics computing framework (BioSig) is being used in this project for cataloging and representing biophysical data such as the movement of labeled AS-ONs into and throughout living cells.

The researchers have successfully demonstrated microscopic digital imaging and quantitation of gene expression in living human breast cancer cells using a fluorescently labeled AS-ON. They have also tracked and manipulated the entry of this agent into cells while logging and documenting microenvironmental conditions. For more information, see http://vision.lbl.gov/Projects/VSOM.

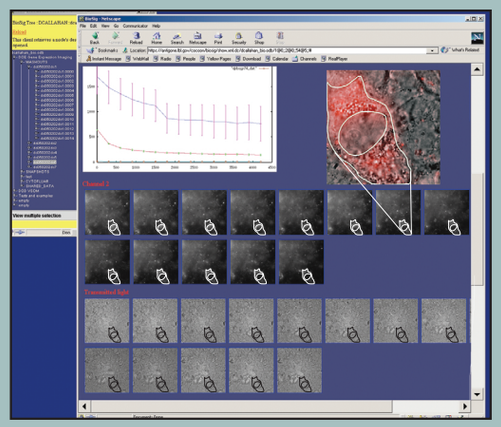
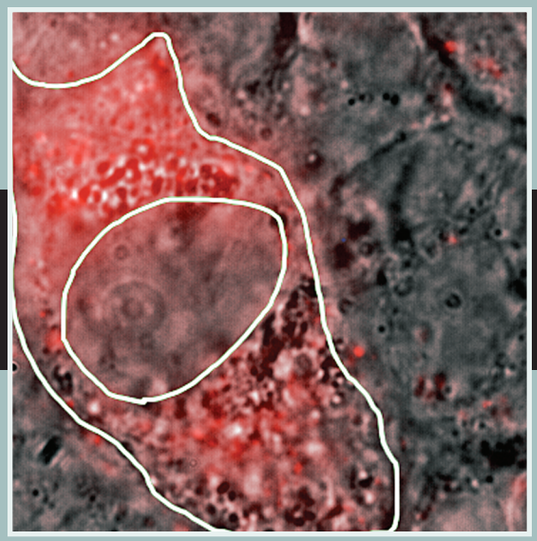


Figure 3. This program has developed new technology such as remote visual-servoing optical microscopy (VSOM) that couples instrumentation with on-line quantitative analysis to elicit specific physiological responses. These responses are archived in a newly developed informatics framework (BioSig) that maps quantitative data to experimental variables. Important data has been generated for the design of AS-ONs and delivery vehicles. Time-lapse images and a corresponding wash-out curve of a fluorescently labeled AS-ON are shown here for a population of cells under observation.

TEID JO 7434

Tools for Molecular Nuclear Medicine

Making Dysfunctional Cells Visible in Humans with Dual-Labeled, Gene-Specific Imaging Agents



BERKELEY LAB
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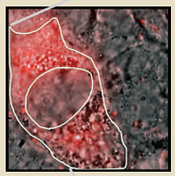
U.S. Department of Energy
Office of Science
Office of Biological and Environmental Research
Medical Sciences Division

One of the most significant advances of the 20th century was nuclear medicine for imaging the functions and structures of organs at the tissue level. Today, researchers at the U.S. Department of Energy's Lawrence Berkeley National Laboratory (Berkeley Lab) are extending nuclear medicine to the molecular level by developing new tools for imaging gene expression. These tools will help medical practitioners diagnose, treat, and even prevent diseases at the level of specific genes within living cells. The goal is to image the activity (or *expression)* of any gene, and to monitor changes in this activity for small subpopulations of cells deep within any human being. Alterations in normal gene activity could indicate the presence of a small tumor or other types of dysfunctional cells. In addition, changes in the activity of a certain gene could indicate a positive or negative response to a recently administered drug.



Specifically, this research is developing imaging agents based on custom-synthesized, artificial DNA and RNA fragments called *antisense oligonucleotides* (AS-ONs). To exploit the potential of these new imaging tools, an infrastructure has been developed for intelligent design and modification of AS-ON agents and delivery vehicles. This infrastructure and a new, multi-step development process leverage DOE's expertise in automation, computer science, medical imaging, and gene technology.

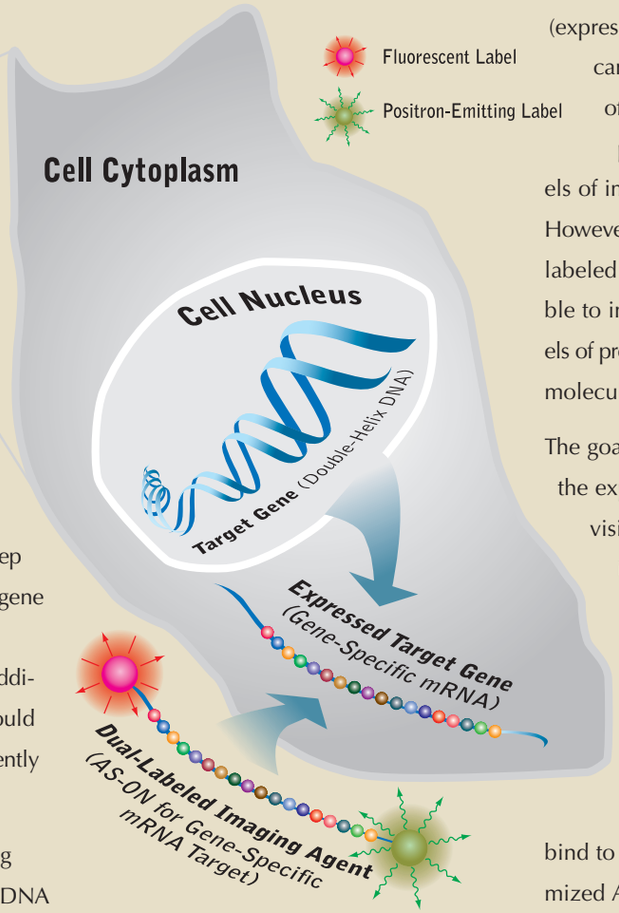


Figure 1. Dual labeling allows one to monitor imaging agent behavior at all stages of the development process. Early stages use a fluorescent label (red circle) and a nonradioactive version of the radiolabel (green circle). Later stages employ a radioactive, positron-emitting version of the radiolabel for PET imaging. Properly designed AS-ONs should accumulate in cells with high levels of the specific mRNA target (inset).

MAKING GENE EXPRESSION VISIBLE

Information encoded in our genes (DNA) is communicated via production (expression) of genetic messengers (mRNA) that carry the instructions for production (expression) of specific proteins. Aberrations in levels of specific mRNA molecules can lead to abnormal, unbalanced levels of associated proteins. It has proven difficult to image levels of important proteins directly. However, properly designed and labeled AS-ONs will make it possible to image the pathological levels of protein-specific mRNA molecules instead.

The goal of this project is to make the expression of specific genes visible via sensitive medical imaging that does not alter normal gene activity. Positron emission tomography (PET) imaging has the sensitivity to detect very small amounts of radiolabeled AS-ONs that bind to target mRNA molecules deep within human beings. Using PET with optimized AS-ONs, it may be possible to measure changing levels of mRNA in humans without perturbing the genetic communication process.

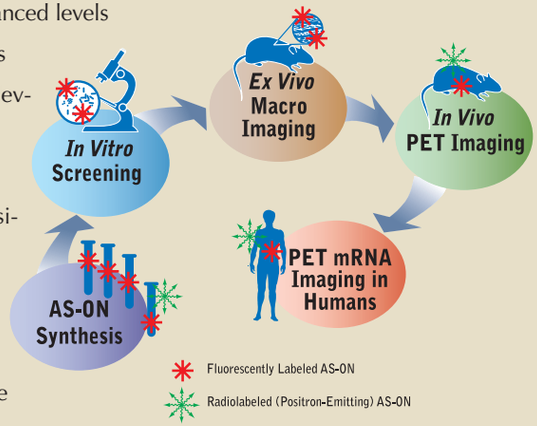


Figure 2. Dual-labeled AS-ONs are first synthesized using automated DNA synthesizers. Then microscope studies select AS-ONs that accumulate properly in living cells *(in vitro* screening); excised tissue is fluorescently imaged to verify proper AS-ON distribution in animals *(ex vivo* macro imaging); and small animal microPET imaging is performed using optimized AS-ONs *(in vivo* PET imaging). The ultimate goal of the project is PET mRNA imaging in humans.

The key to making gene imaging a reality is to make a connection between early, large-scale fluorescence screening, tracking, and design modification experiments and later, smaller-scale PET imaging experiments. To make this connection, the Berkeley Lab research team has developed an iterative protocol for the design, prescreening, and optimization of dual-labeled AS-ON imaging agents (Figure 1). This protocol (Figure 2) produces prescreened and optimized imaging agents and also reduces radioactive waste during the development cycle.